THE ULTRASTRUCTURE OF THE VITELLINE BODY IN THE OOCYTE OF THE SPIDER TEGENARIA PARIETINA

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Vitelline bodies were first observed in 1845 by von Wittich (31) in the oocytes of different species of spiders. In the fifty years that followed, observations of similar organelles multiplied, and by 1900, they had been observed in specimens of almost every class of Metazoa. The various investigators could not, however, reach agreement on the problems presented by this unusual formation and a number of different interpretations were suggested. The most noteworthy of these postulated the existence of an additional nucleus, the migration of a cell of the germinative epithelium into the egg, the existence of a centrosome, a homologous body to the infusorial macronucleus, a male element exerting prefecundation on the egg, a reserve, a formation center for the nutritive elements of the vitellus, an enlarged dictyosome, etc. . . . These bodies are to be found only in the oocytes, and are associated with the formation of the vitellus as has been demonstrated earlier by a number of workers, notably Balbiani (1–3), and more recently by Koch (11), Voinov (29, 30), and Jacquiert (10). For this reason, the term "vitelline body" proposed by Henneguy in 1893 ("corps vitellin") will be kept at least provisionally, as being less ambiguous than "parasome" or "nebenkern" frequently employed for other intracellular formations, and as also having the advantage of defining the function of the body.

Since the development of electron microscopic techniques for biological research, no worker has as yet applied them to the vitelline body in the oocyte of *Tegenaria*.[1] The present work was undertaken in the hope that the electron microscope, in keeping with the rapid progress it is influencing in the fields of cytology, might contribute materially to solving some of the puzzling features presented by this cellular organelle which had so far remained a riddle.

[1] Since the printing of this manuscript, Sotelo and Trujillo-Cenoz (27) have published a study on the oocytes of various South American spiders. The vitelline bodies observed in these species are very different from those studied here in *Tegenaria*. The work of Sotelo and Trujillo-Cenoz will be discussed in a following study on the oocytes of other European species.

Material and Methods

Tegenaria parietina females were dissected immediately after capture. The ovary was cut in small pieces and fixed in Palade's or in Sjöstrand's solution for 30 minutes to 2 hours. Sections, cut with a Porter and Blum microtome, were examined with a Trüb-Taüber KM4, an RCA EMU 2E, or an RCA EMU 3B electron microscope.

The same material was examined supravitally, with or without phase contrast illumination, and after fixation and staining by classical methods (Bouin–hemalum-eosin; Regaud–hematoxylin; Champy–hematoxylin; Carnoy–methyl green–pyronine).

OBSERVATIONS

A. General Morphology (Examination by Classical Methods):

The vitelline body in the oocyte of the spider *Tegenaria parietina* is the most striking organelle of the cell observed supravitally, by classical light microscopy as well as by phase contrast microscopy. It is a yellowish spherical body clearly outlined, which contrasts sharply with the cytoplasmic background, even when the latter is full of vitelline platelets. The spherical oocyte encloses two spheres of considerable size: the germinal vesicle and the vitelline body. As a rule, their respective diameters are $\frac{2}{5}$ and $\frac{1}{3}$ that of the oocyte. Both are excentric, and are separated by a certain amount of cytoplasm. The vitelline body is sometimes nearer the chorion than the nucleus, hence the term "paranucleus," sometimes applied to it, is morphologically unsuitable.

The vitelline body in the mature oocyte reaches 80 microns. The observations *in vivo* and after staining allow 4 different zones to be distinguished:

1. *A central zone* in which particles, vesicles of variable shape and refractility or irregular membranous folds may be observed.
2. *A lamellar medial zone*, by far the most important in size. This is composed of concentric, more or less undulating spherical membranes between which optically transparent pockets may frequently be distinguished.
3. *An optically "empty" zone*, of slight but variable width (at most a few microns).
4. *A vesicular external zone* where closely packed vesicles are extremely numerous. These have a small diameter, considerably below 1 μ on an average, and they sometimes show a rough alignment. Methyl green–pyronine staining indicates basophilia in this zone.

No membrane can be distinguished enclosing the vitelline body. The customary cytoplasm and its organelles are found beyond the vesicles, without any sudden transition.

B. Ultrastructure

1. The Central Nodule.—The electron microscope reveals that in mature oocytes, which alone have been studied here, the central zone of the vitelline body does not differ essentially from the lamellar zone which surrounds it. The

only differences are: (1) the lamellar material is less abundant and, inversely, the granular areas and the vesicles are more frequent; (2) the concentric disposition of the lamellae is less regular; their outline undulates and, sometimes, forms whirls and loops (Fig. 1).

2. *The Lamellar Zone.*—It consists of numerous concentric lamellae which are stacked and separated by a granular substance and various interposed formations.

(*a*) *The Stacks of Lamellae.*—Each "lamella" seen with the light microscope is, at the level of the electron microscope, composed of a *stack of lamellae.* These are parallel sheets between two and twenty in number. Very often, such a stack is divided into two or more groups which may join up, or link up with neighbouring stacks (Figs. 2 and 3). Though they are in general arranged concentrically (Fig. 1), their outline is frequently irregular, in the form of curves and even rings (Fig. 3).

The thickness of a single sheet is 45 A. The average distance between two adjacent sheets is 50 to 60 A (Fig. 5). Their extent in other dimensions seems to be considerable. While it is not uncommon to observe the sudden termination (edge) of a sheet in the sections, more often a gradual disappearance is observed with the changing direction of the sheets with respect to the plane of the section. It is, therefore, difficult to evaluate the extent of the membranes, but it is certain that they generally measure several microns, perhaps several tens of microns.

When a sheet ends in the middle of a stack, the adjacent sheets adopt abruptly the usual separation, in the manner encountered in marginal dislocations in crystalline lattices (Fig. 2, arrow).

(*b*) *The Interlamellar Substance.*—Various cytoplasmic components are found between the stacks of lamellae. The most frequent is a finely granular mass. This substance is composed of very closely packed granules, of moderate density, and about 50 to 60 A in diameter (Fig. 2). Some have a light center. Very often, near the lamellae, they show an alignment giving a gradual transition from grains to lamellae (Fig. 7).

In the light areas, another granular substance, more opaque and of notably larger grain (100 to 120 A), is to be found (Figs. 3 and 4). The grains here are sometimes aligned and are more abundant in the peripheral zone of the lamellae.

Various vesicles are scattered in the background. Their size varies from $\frac{1}{20}\ \mu$ to 1 μ. They are found either in the particulate masses or lying against the lamellae. The walls of these vesicles flatten against the last sheet and completely resemble it (Fig. 2). Most of the vesicles appear to have a clear homogenous interior; others show various structures, such as granules, granular or cloudy masses, spherules, or tubules. The structure of some of these elements suggests a relationship with mitochondria. In no case, however, has it been possible to discover mitochondria with normal morphology in the lamellar zone.

3. The Zone Optically "Empty" in the Light Microscope.—Essentially, this zone consists of a finely granular substance accumulated in abundant masses at the periphery of the lamellae. Near its limits, the lamellar zone contains a reduced number of lamellae, and, correspondingly, more granular masses. The lamellar periphery no longer presents thick layers, but merely a small number of sheets embedded in liberal amounts of granules (Fig. 6). Locally, the latter form clusters of several microns in thickness. This zone therefore is a zone of transition.

4. The Vesicular Zone.—Here again, the transition is gradual. The granular zone loses its compactness, being hollowed by anfractuosities which contain the inclusions described below. The zone then appears as a very open network with elongated meshes. Then, as the transformation progresses, only scattered islands of the granular substance remain.

The vesicular zone contains ergastoplasm, dense particles, mitochondria, and Golgi material (Figs. 8 to 10).

The ergastoplasm, which is unusually abundant, is composed of cavities and dense particles. The cavities are rounded or oval, more occasionally with irregular outlines. They are formed by a membrane with the opaque particles attached to it.

The dense particles (100 to 150 A) are also frequently found free in the hyaloplasm, isolated or in small groups. Sometimes they seem to be aligned along very fine membranes which are difficult to distinguish (Figs. 9, 10), or attached to the granular masses.

Large dark spheres without a limiting membrane, about 200 to 250 mμ in diameter, are quite frequently found inside the ergastoplasmic cavities. These bodies may show some heterogeneity in the form of less opaque areas (Figs. 8 to 10).

The circular mitochondrial profiles measure 0.2 to 0.5 μ; their occasional cristae are tubular and usually short.

In the young germ cell, mitochondria are frequently elongated with numerous cristae and a dense matrix. In the peripheral cytoplasm of the mature oocyte, they are usually round (0.2 to 1.0 μ) with a dense matrix containing tubules. Some of the mitochondria, again elongated but moniliform, seem to divide up into rounded elements (Fig. 11).

The Golgi apparatus consists of a collection of small spherical vesicles of 40 to 80 mμ in diameter (Fig. 9). Their interior, as well as the cytoplasmic background which surrounds them, is somewhat opaque, so that these zones are much darker than the rest of the cytoplasm. The number of vesicles in one section may reach 200 in a single group. But much smaller groups and even solitary vesicles do exist. When in groups, much larger vesicles can be found among them, whose shape and structure are comparable to those of the ergastoplasmic vesicles, but without their attached particles. No "double membranes" have been found in these Golgi zones.

In the young cell, the Golgi apparatus is concentrated and has an abundant lamellar component (Fig. 12). In the peripheral cytoplasm of the mature oocyte, it is dispersed to form Golgi bodies in which the lamellar material, in the shape of stacked flattened vesicles, breaks up into beads at its limits.

DISCUSSION

The two remarkable features of the ultrastructure of the vitelline body are the regular stacking of the sheets in the lamellar zone and the existence around this lamellar sphere of a specialized cytoplasmic area. Some of the morphological problems presented by these two regions will be discussed. The physiology and cytochemistry will form the object of a separate study.

1. The Structure of the Lamellar Zone.—From the periphery towards the center the vitelline body shows:

(*a*) a great abundance of the dense particles (100 to 120 A) described by Palade (14);

(*b*) their almost complete absence from the surface of the granular masses, which contain only very few such particles;

(*c*) the alignment of small particles (50 to 60 A) in the granular masses;

(*d*) the regular stacking of many sheets.

This arrangement poses two questions: (1) What becomes of the dense particles and (2) is there a relation between the small particles and the sheets?

The presence of the dense particles in the granular masses suggests that these particles—and perhaps the ribonucleic acids which they contain (19, 20)—participate in the formation of these granular masses. At this time, however, it is impossible to say whether or not the small particles of 50 A are formed from the dense particles.

It would appear that the small particles represent an essential component of the sheets. There are three arguments in favour of this hypothesis: (1) the particles show a marked tendency to arrange themselves in parallel lines; (2) the continuation of a line of particles into a sheet can sometimes be observed; (3) the moniliform appearance of some sectioned sheets suggests their granular origin.

The above hypothesis presupposes that the vitelline body increases from the outside, but the inverse can also be postulated and has already been discussed. Koch (11) has stated that the new lamellae appear in the interior. On the contrary, Jacquiert (10), who observed their formation during the growth of the oocyte, saw them originate on the surface and his arguments are most convincing. It has not been possible to confirm them with the electron microscope.

The fine structure and the regular lamellar arrangement recalls that of myelin sheaths (Sjöstrand (26), Robertson (22), Fernández-Morán (5)), retinal rods (Sjöstrand (25)), and chloroplasts (Steinmann and Sjöstrand (28), Hodge *et al* (8)). The thickness of the membranes—45 A—is in agreement with that indicated among others by Robertson (50 A), Fernández-Morán (40 A) for the

lamellae of myelin sheaths, and Hodge (35 A) for the lamellae of chloroplasts. As to the interlamellar spacing (60 A), it is somewhat below the values encountered in the structures mentioned and it is not divided by an intermediate band as is the case for myelin sheaths (Sjöstrand, Fernández-Morán) and the grana of chloroplasts (Hodge).

It has been postulated (8, 18, 24) that such laminated structures represent lipoprotein systems in which the dark lines correspond to layers of proteins, with lipide molecules between them, whose long axes are perpendicular to the plane of the lamellae. This parallel arrangement of molecular layers may explain the birefringence of the vitelline body observed by Parat and Jacquiert (21), and the local readjustments of these molecular layers similar to marginal dislocations.

2. The Vesicular Zone.—This zone is characterized by the disposition of the dense particles described by Palade (14), the appearance of its ergastoplasm, mitochondria, and Golgi apparatus.

It is not our intention here to discuss the problem of ergastoplasm, which is not truly relevant. Readers interested therein are refered to the most recent works on the subject (18, 15, 16, 7). The ergastoplasm consists of the association of two components: (1) membranes which are part of the endoplasmic reticulum; (2) dense particles described by Palade (14), named "ribonucleoprotein particles" by most authors now that their high content in RNA and protein is well established.

The abundance of ergastoplasm and dense particles reflects the intense metabolic activity of the zone. Numerous writers (*cf.* the review by Leslie (12)) have in fact shown that increased ergastoplasm is always linked with an abundant synthesis of proteins.

A large number of dense particles are scattered through the hyaloplasm or attached to extremely tenuous membranes. The ergastoplasmic cavities are not flattened but dilated, with few particles attached. It is still difficult to interpret the significance of these peculiar aspects. The predominance of free dense particles has been noted in cells of rapid proliferation (Palade (14)), in hepatic embryonic cells (Howatson and Ham (9)), tumor cells (Howatson and Ham (9)), regenerating cells (Oberling and Rouiller (13)), sarcomatous cells in tumors of rapid growth (Rouiller *et al.* (23)), and in the silk-glands of the silkworm (Bernhard *et al* (4)); all these are examples of high synthesizing activity in the cytoplasm.

A feature which deserves to be emphasized is the presence in some of the ergastoplasmic cavities of opaque spherical bodies, 200 to 250 mμ in diameter. The presence of intraergastoplasmic bodies is surprising. Hitherto, only a clear homogenous content has been reported in the cavities of the ergastoplasm, with the exception of Palade (17). His study of the pancreas revealed "intracisternal granules" which in size, shape, and opacity, correspond to the bodies described above. He postulated a relationship between them and the zymogen

granules. Their occurrence in cells very different from those of the pancreas assigns to these bodies a greater significance in terms of general cellular metabolism. It is interesting to notice that these intraergastoplasmic bodies exist throughout the oocyte without morphologic variation.

Finally, the unusual appearance of the mitochondria and of the Golgi apparatus of this peculiar cytoplasmic portion is of interest. Their simplified morphology has already been mentioned: the mitochondria, which are rounded, possess few cristae; the Golgi bodies have no lamellar element and few clear vacuoles. It would appear that the characteristics of the chondriome and of the Golgi complex in the vitelline body are connected with the function of this cytoplasmic region. Limited knowledge permits only conjecture at this point. Grassé (6) has interpreted the peripheral breaking up into beads ("perlage") of the Golgi vesicles as secretory droplets. Wohlfarth-Bottermann (32) described the extrusion of cristae from mitochondria, and here again the writer spoke of a secretion. If these interpretations are correct, the mitochondria and the Golgi apparatus discussed would be at the highest level of their secretory activity. Their products, together with the ribonucleic acids of the dense particles, might play a part in the formation of the vitelline body. "Ghosts" of such mitochondrial and Golgi origin might thus be found trapped between newly formed lamellae, which in turn would account for some of vesicles described in the lamellar zone.

The vitelline body therefore would appear to represent a phase during which material is accumulated in paracrystalline formation. Subsequently it might be expected to play a part in the production of the vitelline platelets and the metabolism of the newly fertilized egg.

SUMMARY

The vitelline body in the mature oocyte of the spider *Tegenaria parietina* is composed of 4 different zones.

1. The central zone contains granular areas, vesicles, and a few lamellae.

2. The lamellar zone consists of numerous concentric lamellae. These sheets, 45 A in thickness, are stacked in groups. The fine structure and the regular arrangement recall those of myelin sheets, retinal rods, and chloroplasts. Between the stacks of lamellae, finely granular masses and various vesicles are to be found.

3. The "zone of transition" consists of a finely granular substance accumulated in abundant masses. This substance is composed of very closely packed granules about 50 to 60 A in diameter. Very often, near the lamellae, the granules show alignment giving a gradual transition from grains to lamellae.

4. The vesicular zone contains ergastoplasm, dense particles, mitochondria, and Golgi material. It is suggested that the peculiar ultrastructure of these cytoplasmic components may be related to an intense metabolic activity.

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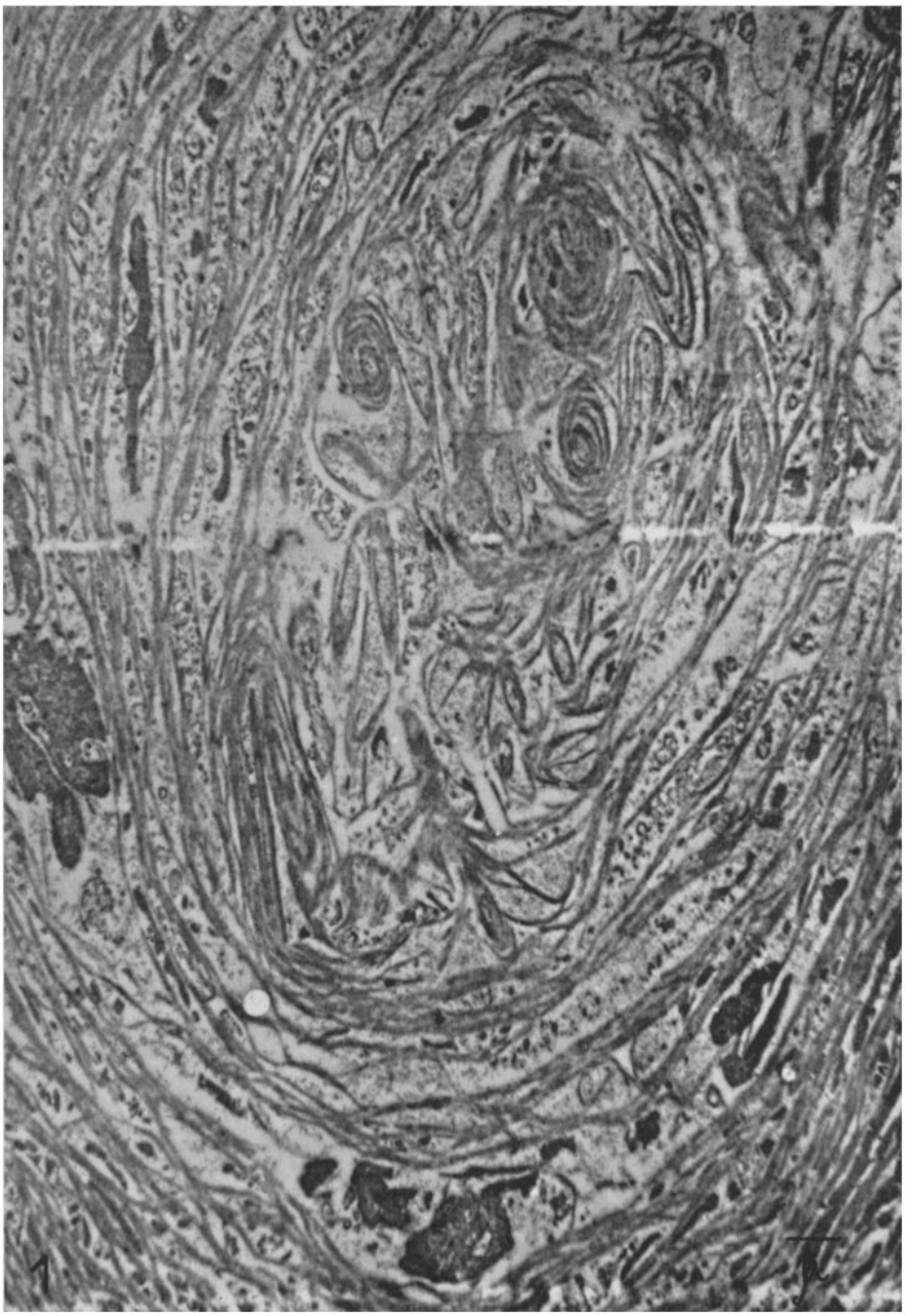
EXPLANATION OF PLATES

PLATE 291

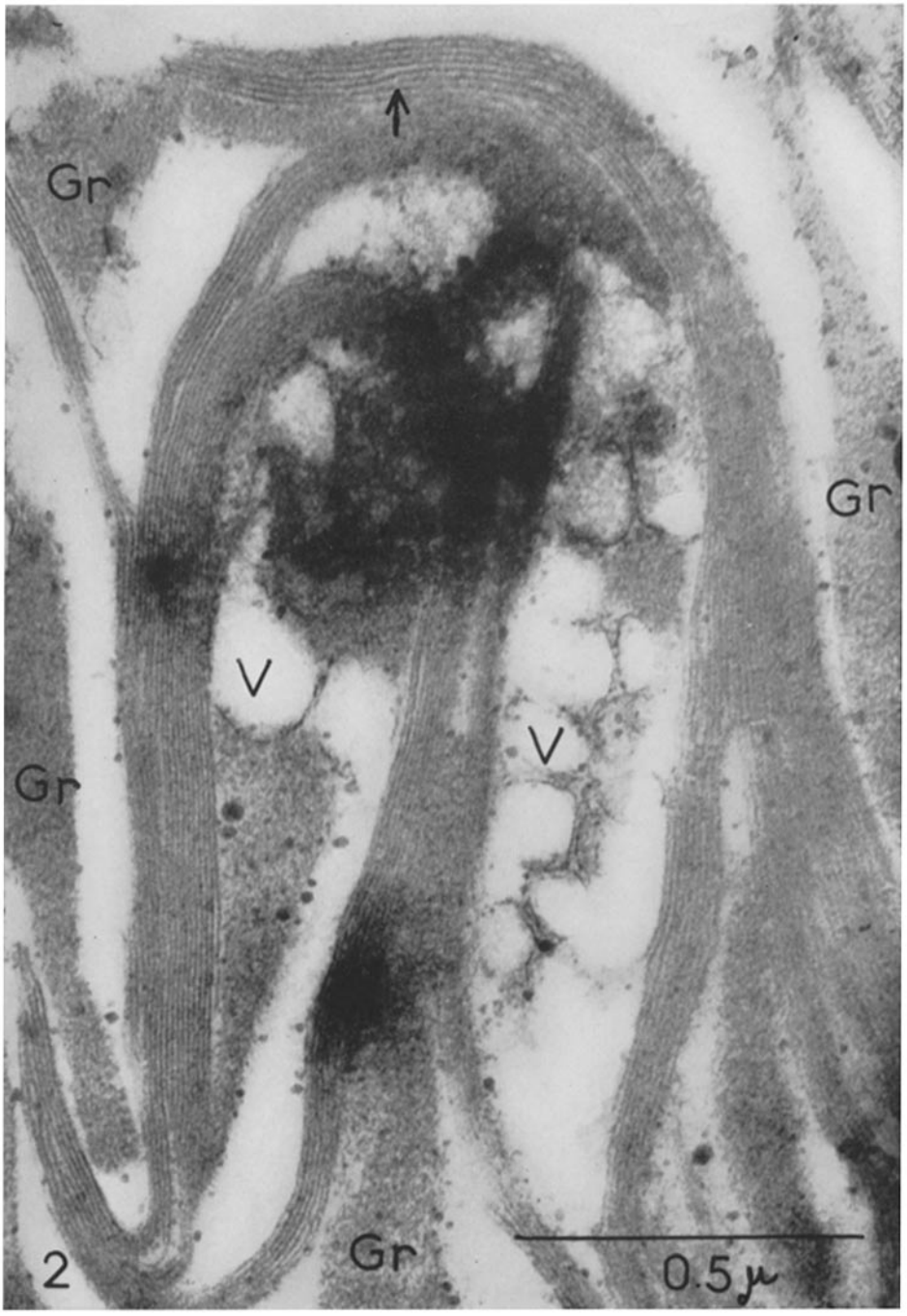
FIG. 1. Central zone of the vitelline body. The stacks of lamellae are more loosely packed than at the periphery. They describe numerous curves and loops. The interlamellar material (vesicles, granules, granular masses) is abundant. × 7,500.



(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 292

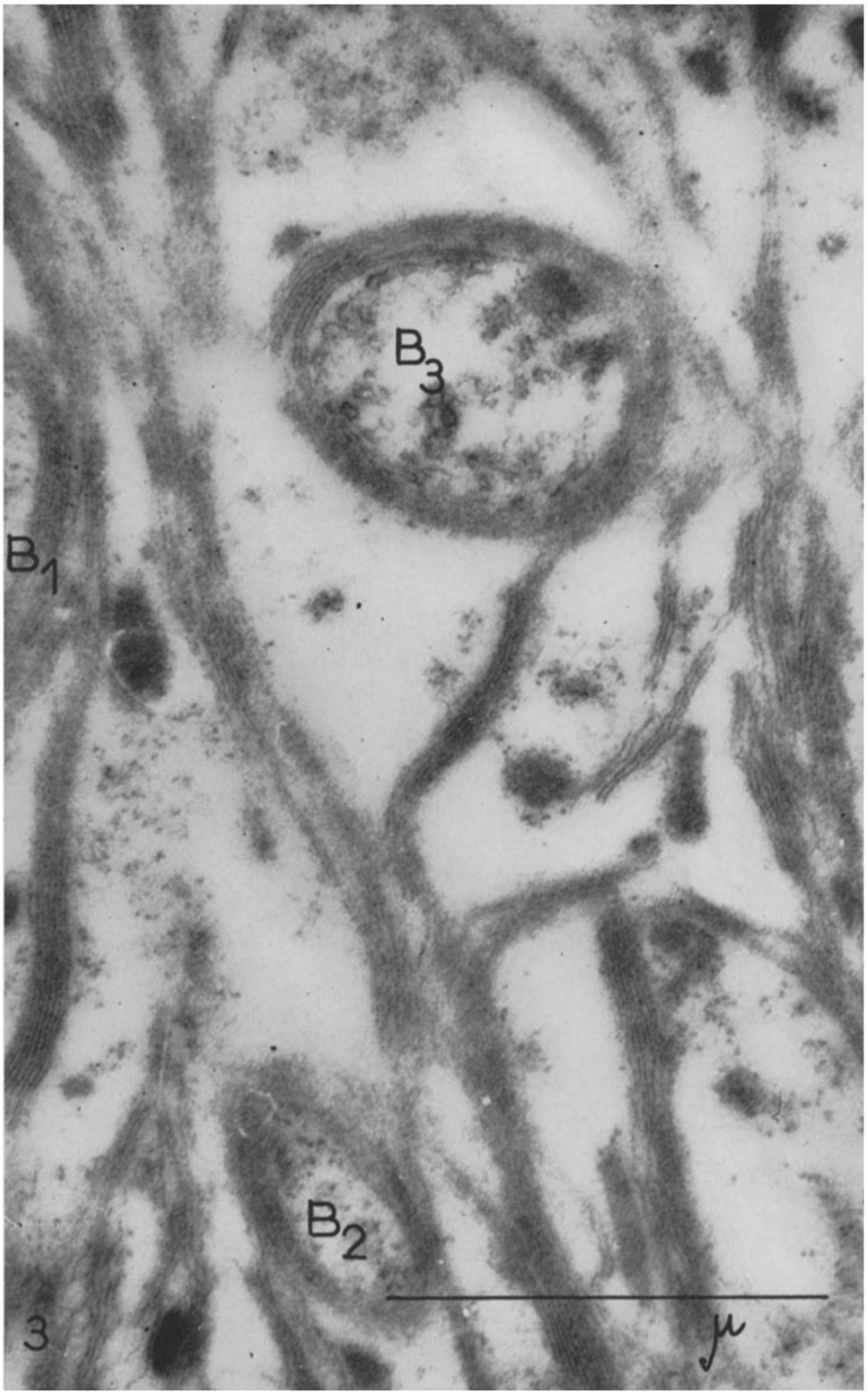
FIG. 2. Lamellar zone. The lamellae are parallel. Between the stacks of lamellae, vesicles, *V*, and a finely granular substance (50 A), *Gr*. Arrow points to abrupt ending of membranes in the midst of a pile (reminiscent of marginal dislocations). × 96,000.



(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 293

FIG. 3. Lamellar zone. Note the irregular outline of the stacks of lamellae dividing into several groups, which may rejoin or unite with neighbouring stacks. The loops have a granular (B_1, B_2) or vesicular (B_3) content. × 64,000.

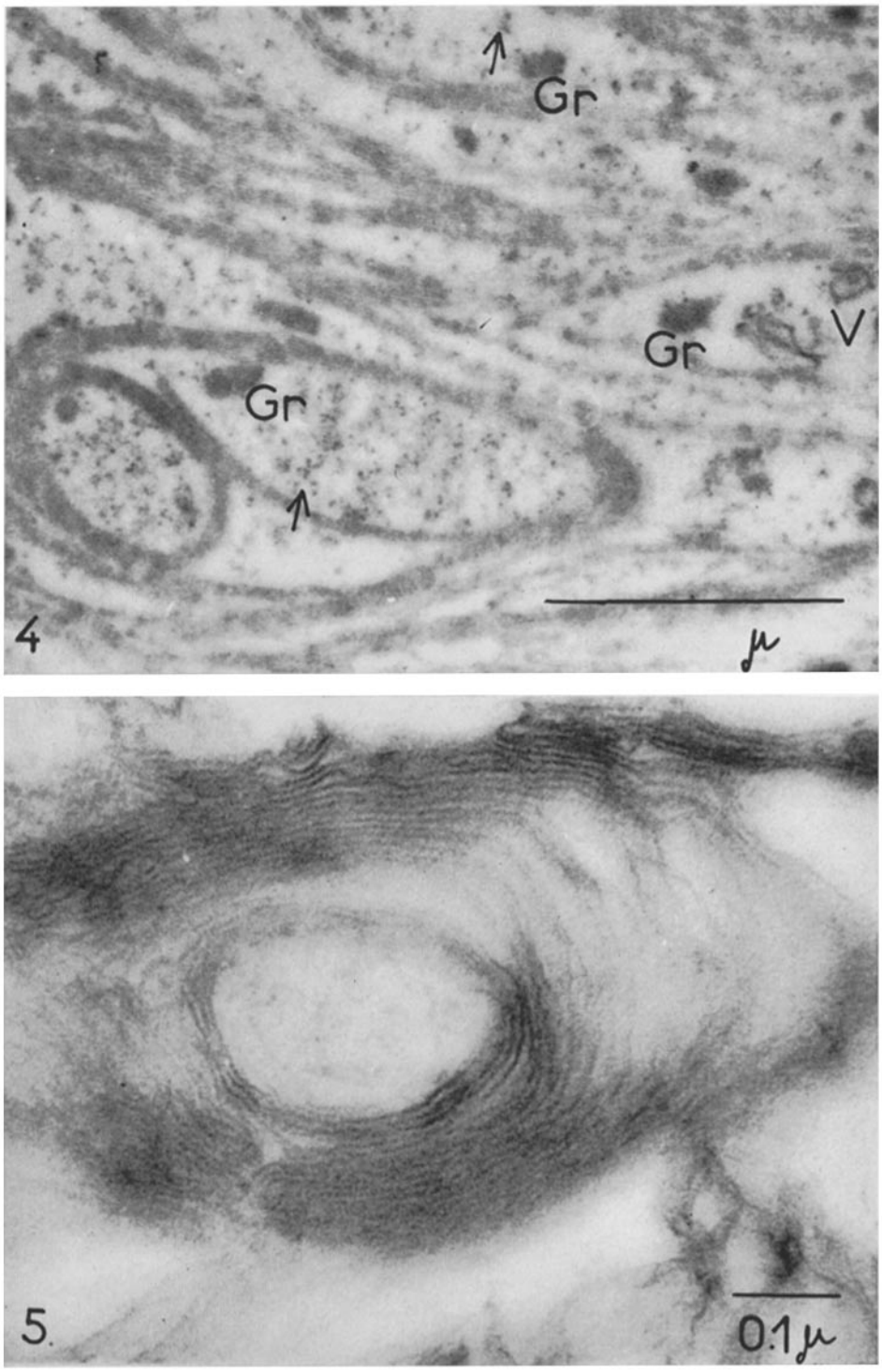


(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 294

FIG. 4. Lamellar zone. Dense particles (arrows); finely granular substance, *Gr*; unidentified vesicles, *V*. × 43,000.

FIG. 5. Lamellar zone. Loop-like appearance due to the section being near the top of a lamellar cone (enlargement of Fig. 8). At its periphery, the cone enlarges, and the membranes are sectioned obliquely. × 144,000.

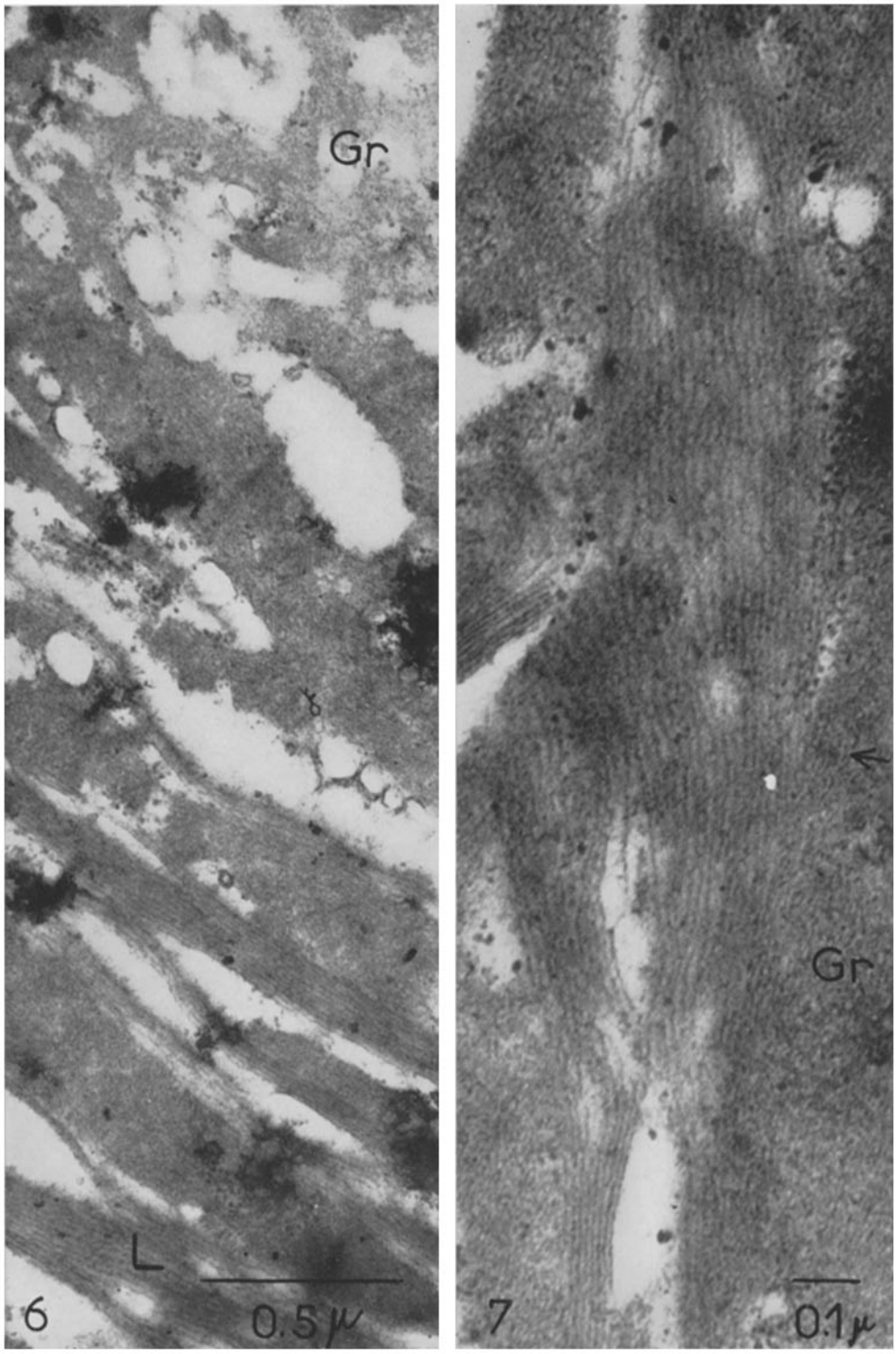


(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 295

FIG. 6. Zone of transition. Change from fine granular masses, *Gr*, to lamellar zone, *L*. × 57,000.

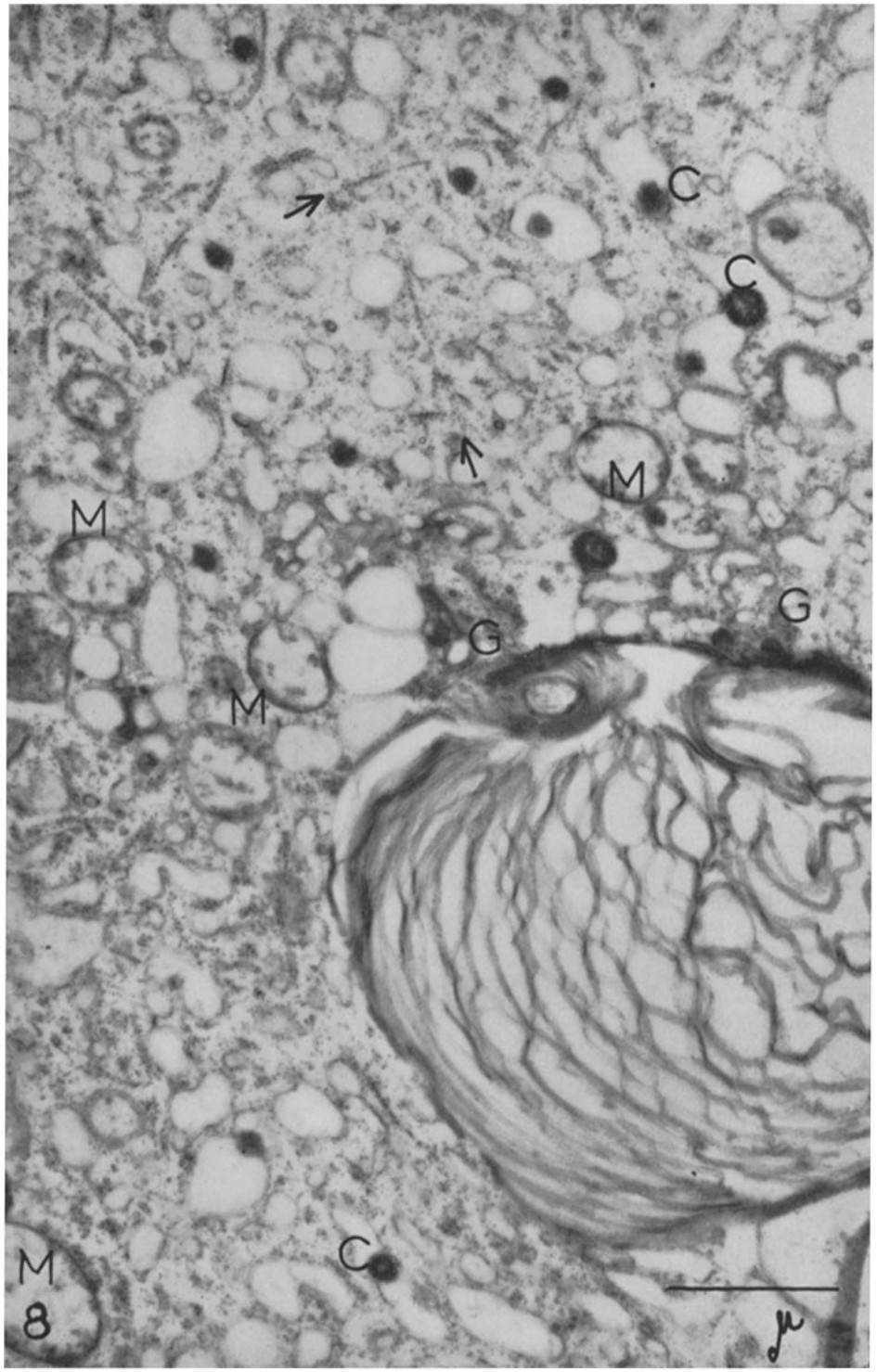
FIG. 7. Lamellar zone. In the granular masses (*Gr*), the particles are often aligned (arrow). × 93,000.



(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 296

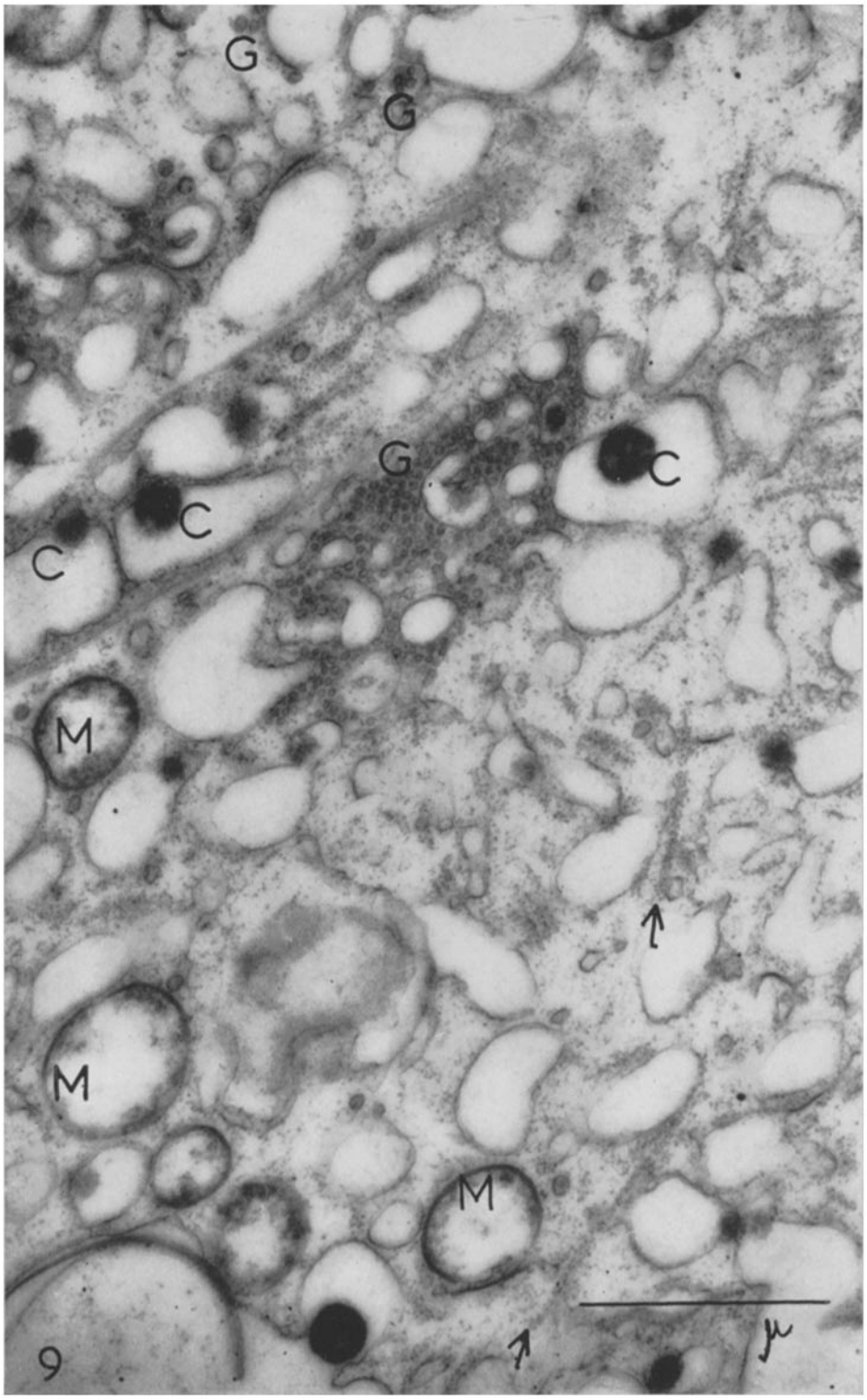
FIG. 8. Lamellar and vesicular zones. A cone of lamellae projected into the vesicular zone is sectioned near its top (the central part is enlarged in Fig. 5). Notice the lack, in this place, of the fine granular material, the presence of intraergastoplasmic bodies *C*, and the abundance of dense particles sometimes lined in rows (arrows). Golgi microvesicles, *G*, are loosely packed or isolated. *M*: mitochondria. × 25,000.



(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 297

FIG. 9. Vesicular zone. In the center, a Golgi body, composed only of microvesicles. Here and there, isolated microvesicles, *G*. Arrows: dense particles aligned in rows. *C*: intraergastoplasmic bodies. *M*: mitochondria. × 36,000.



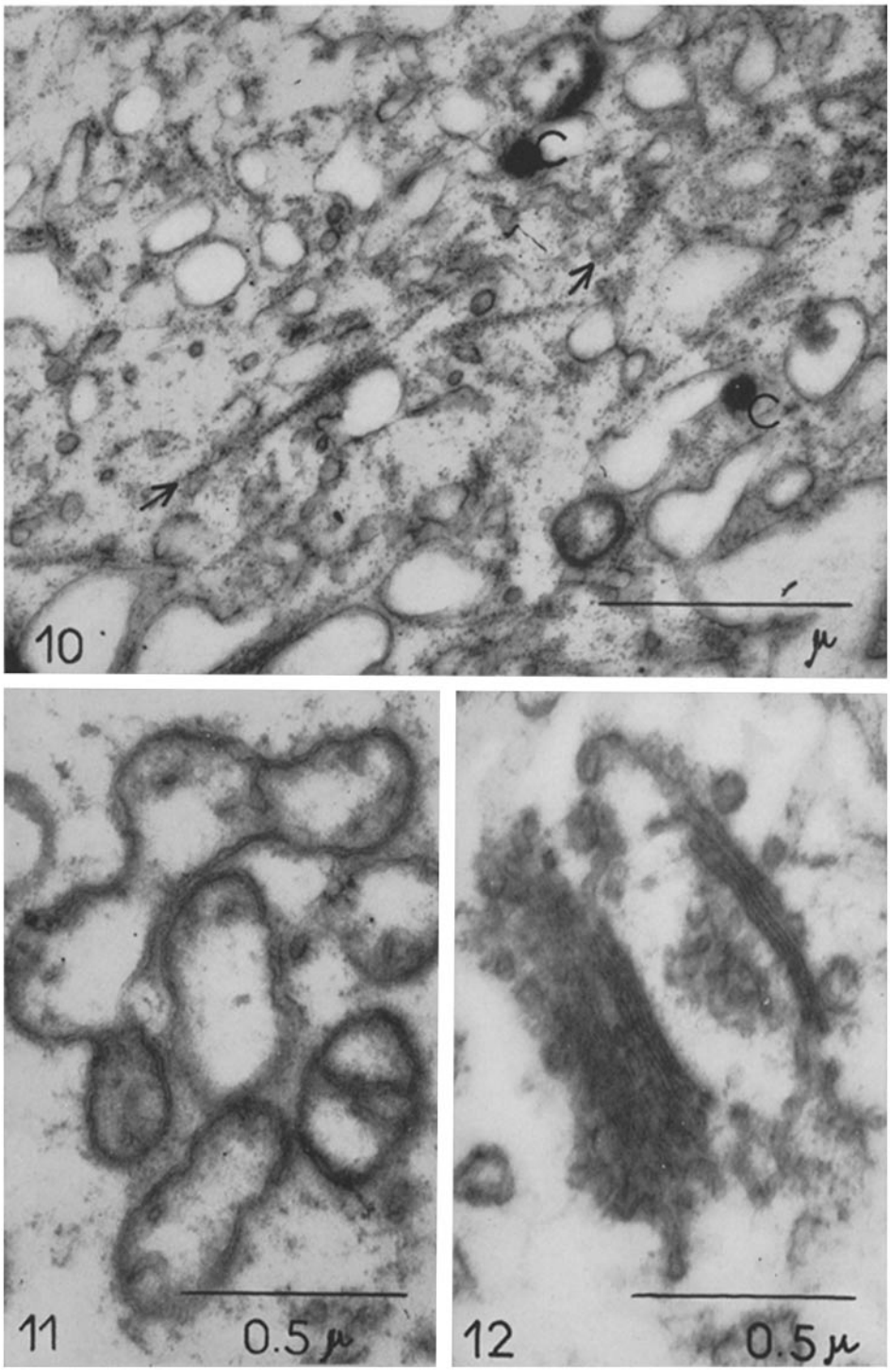
(André and Rouiller: Vitelline body in oocyte of spider)

PLATE 298

FIG. 10. Vesicular zone. Alignment of dense particles (arrows). Intraergastoplasmic bodies, *C*. × 36,000.

FIG. 11. Mitochondria, in the cytoplasm of a young oocyte. One of them, moniliform, appears to break up into round mitochondria. × 64,000.

FIG. 12. Golgi zone, in the cytoplasm of a young oocyte. The edges of the vesicles break up into beads (microvesicles). × 64,000.



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